

WHAT IS CLAIMED IS:

1. A method for detection of at least one allele of a genetic locus comprising amplifying genomic DNA with an intron-spanning primer pair that defines a DNA sequence, said DNA sequence being in genetic linkage with said genetic locus and containing a sufficient number of intron sequence nucleotides to produce an amplified DNA sequence characteristic of said allele.
2. The method of Claim 1 wherein said amplified DNA sequence includes at least about 300 nucleotides corresponding to intron sequences.
3. The method of Claim 1 wherein said intron sequence is adjacent to an exon encoding said allele.
4. The method of Claim 1 wherein said amplified DNA sequence is characteristic of at least one nonadjacent allele.
5. The method of Claim 1 wherein said amplified DNA sequence is characteristic of at least one adjacent allele and at least one nonadjacent allele.
6. The method of Claim 5 wherein said amplified DNA sequence includes at least about 1,000 nucleotides corresponding to intron sequences.
7. A method for detection of at least one allele of a genetic locus comprising:
 - a. amplifying genomic DNA with an intron-spanning primer pair that defines a DNA sequence, said DNA sequence being in genetic linkage with said allele and containing a sufficient number of intron sequence nucleotides to produce an amplified DNA sequence characteristic of said allele; and

- 1 b. analyzing said amplified DNA sequence to
2 detect the presence of a genetic variation in
3 said amplified sequence.
- 4 8. The method of Claim 7 wherein said variation in
5 said amplified DNA sequence is a variation in the
6 length of the primer-defined amplified DNA
7 sequence.
- 8 9. The method of Claim 7 wherein said variation in
9 said amplified DNA sequence is a change in the
10 presence of at least one restriction site in the
11 primer-defined amplified DNA sequence.
- 12 10. The method of Claim 7 wherein said variation in
13 said amplified DNA sequence is a change in the
14 location of at least one restriction site in the
15 primer-defined amplified DNA sequence.
- 16 11. The method of Claim 7 wherein said variation in
17 said amplified DNA sequence is a substitution of
18 at least one nucleotide in the primer-defined
19 amplified DNA sequence.
- 20 12. The method of Claim 7 wherein said genetic locus
21 is a major histocompatibility locus.
- 22 13. The method of Claim 7 wherein said allele is
23 associated with a monogenic disease.
- 24 14. The method of Claim 13 wherein said monogenic
25 disease is cystic fibrosis.
- 26 15. The method of Claim 7 wherein at least about 70%
27 of said primer-defined amplified DNA sequence
28 corresponds to intron sequences.
- 29 16. The method of Claim 7 wherein said primer-defined
30 amplified DNA sequence is from 300 to 500
31 nucleotides in length.
- 32 17. A method for producing RFLP fragments for an HLA
33 locus of an individual comprising the steps of:
34 a. amplifying genomic HLA DNA from said
35 individual with a primer pair specific for

- 1 said HLA locus under conditions suitable to
2 produce an amplified DNA sequence; and
3 b. producing a digest by combining said
4 amplified DNA sequence with at least one
5 endonuclease that cleaves said amplified DNA
6 sequence to yield a set of fragments having
7 distinctive fragment lengths.
- 8 18. The method of Claim 17 additionally comprising the
9 step of producing RFLP patterns from said digest.
- 10 19. The method of Claim 17 wherein said primers define
11 a DNA sequence that contains all exons that encode
12 allelic variability associated with said HLA
13 locus.
- 14 20. A method for producing RFLP fragments for an HLA
15 locus of an individual comprising the steps of:
16 a. amplifying genomic HLA DNA from said
17 individual with a primer pair specific for
18 said HLA locus under conditions suitable to
19 produce an amplified DNA sequence, said
20 primers defining a DNA sequence that contains
21 all exons that encode allelic variability
22 associated with said HLA locus; and
23 b. producing a digest by combining said
24 amplified DNA sequence with at least one
25 endonuclease that cleaves said amplified DNA
26 sequence to yield a set of fragments having
27 distinctive fragment lengths.
- 28 21. A method for producing RFLP patterns for an HLA
29 locus of an individual comprising the steps of:
30 a. amplifying HLA DNA from said individual with
31 a primer pair specific for said HLA locus
32 under conditions suitable to produce an
33 amplified DNA sequence, said primers being
34 located in intervening sequence I and in
35 intervening sequence III when said HLA locus
36 is a Class I locus and in intervening

1 sequence I and in intervening sequence II
2 when said locus is a Class II locus;
3 b. producing a digest by combining said
4 amplified DNA sequence with at least one
5 endonuclease that cleaves said amplified DNA
6 sequence to yield a set of fragments having
7 distinctive fragment lengths; and
8 c. producing RFLP patterns from said digest.
9 22. The method of Claim 21 wherein said amplification
10 comprises:
11 a. combining an HLA-locus specific primer pair
12 with HLA DNA from said individual under
13 hybridizing conditions for a period of time
14 sufficient for each primer in said primer
15 pair to produce an extension product which,
16 when separated from its complement, can serve
17 as a template for synthesis of the extension
18 product of the other primer to produce a
19 mixture;
20 b. treating said mixture under denaturing
21 conditions to separate the primers from their
22 extension products;
23 c. treating said mixture with said HLA locus-
24 specific primer pair such that a primer
25 extension product is synthesized using each
26 of the templates produced in step (b) as a
27 template, resulting in amplification of the
28 HLA DNA; and
29 d. repeating steps (b) and (c) to produce an
30 amplified DNA sequence.
31 23. The method of Claim 21 wherein a second primer
32 pair specific for said HLA locus is also used to
33 amplify said HLA DNA.
34 24. The method of Claim 21 wherein producing said RFLP
35 fragment pattern comprises:

- 1 a. combining said amplified DNA sequence with at
2 least one endonuclease that cleaves said
3 amplified DNA sequence to yield a set of
4 fragments having distinctive fragment
5 lengths;
6 b. separating said fragments based on the length
7 of the fragments to produce separated
8 fragments; and
9 c. visualizing said separated fragments to
10 produce RFLP fragment patterns.
- 11 25. The method of Claim 24 wherein said fragments are
12 separated using gel electrophoresis and visualized
13 using a nucleotide-specific stain.
- 14 26. A method for determining whether DNA in a sample
15 is from a particular individual comprising the
16 steps of:
17 a. amplifying DNA from said individual and DNA
18 from said sample with a primer pair specific
19 for an HLA locus under suitable conditions to
20 produce an amplified DNA sequence from said
21 individual and from said sample, said primers
22 being located in intervening sequences I and
23 III for an HLA Class I locus and in
24 intervening sequences I and II for a Class II
25 locus;
26 b. combining said amplified DNA sequence from
27 said individual and said amplified sample DNA
28 from said sample with at least one
29 endonuclease that cleaves said amplified DNA
30 sequence into a plurality of cleaved
31 sequences of sufficiently different lengths
32 to distinguish between alleles of said HLA
33 locus for a period of time sufficient for
34 digestion of said amplified DNA to produce a
35 digest; and

- 1 c. comparing restriction fragment length
2 polymorphic patterns produced by said digest
3 from said individual and from said sample.
- 4 27. A method for determining whether an individual is
5 the father of a child comprising the steps of:
6 a. amplifying DNA from said individual, DNA from
7 said child and DNA from said child's mother
8 with a pair of primers specific for an HLA
9 locus under suitable conditions to produce
10 amplified DNA sequences, said primers being
11 located in intervening sequences I and III
12 for an HLA Class I locus and in intervening
13 sequences I and II for a Class II locus;
14 b. combining said amplified DNA sequence from
15 said individual and said amplified sample DNA
16 from said child with at least one
17 endonuclease that cleaves said amplified DNA
18 sequence into a plurality of cleaved
19 sequences of sufficiently different lengths
20 to distinguish between alleles of said HLA
21 locus to produce a digest; and
22 c. comparing restriction fragment length
23 polymorphic patterns produced by said digest
24 from said individual, from said child's
25 mother and from said child.
- 26 28. An HLA locus-specific primer selected from the
27 group consisting of a Class I locus-specific
28 primer, a Class I A locus-specific primer, a Class
29 I B locus-specific primer and a Class I C locus-
30 specific primer.
- 31 29. The HLA locus-specific primer of Claim 28 wherein
32 said primer has a sequence corresponding to at
33 least 15 consecutive nucleotides selected from the
34 group consisting of CATGTGGCCATCTTGAGAATGGA;
35 GCCCGGGAGATCTACAGGCGATCA; CGCCTCCCTGATCGCCTGTAG;
36 CCAGAGAGTGACTCTGAGG; CACAATTAAGGGAT;

1 TCCCCGGCGACCTATAGGAGATGG; CTAGGACCACCCATGTGACCAGC;
 2 ATCTCCTCAGACGCCGAGATGCGTCAC;
 3 CTCCTGCTGCTCTGGGGGGCAG; ACTTTACCTCCACTCAGATCAGGAG;
 4 CGTCCAGGCTGGTGTCTGGGTTCTGTGCCCCT;
 5 CTGGTCACATGGGTGGTCCTAGG;
 6 CGCCTGAATTTTCTGACTCTTCCCAT;
 7 ATCCCCGGGAGATCTACAGGAGATG; AACAGCGCCCATGTGACCATCCT;
 8 CTGGGGAGGCGCCGCGTTGAGGATTCT;
 9 CGTCTCCGCAGTCCCGGTTCTAAAGTTCCCAGT;
 10 ATCCTCGTGCTCTCGGGA; TGTGGTCAGGCTGCTGAC;
 11 AAGGTTTGATTCCAGCTT;
 12 CCCCTTCCCCACCCAGGTGTTCTGTCCATTCTTCAGGA;
 13 CACATGGGCGCTGTTGGAGTGTCTG; GTGAGTGCGGGGTCTGGGAGGGA;
 14 CACCCACCGGGACTCAGA; TGGCCCTGACCCAGACCTGGGC;
 15 GAGGGTCGGGCGGGTCTCAGC; CTCTCAGGCCTTGTTTC;
 16 CAGAAGTCGCTGTTCC; TTCTGAGCCAGTCCTGAGA;
 17 TTGCCCTGACCACCGTGATG; CTCCTGCTTGTCATCTTCA;
 18 CCATGAATTTGATGGAGA; ACCGCTGCTACCAATGGTA;
 19 CCAAGAGGTCCCCAGATC; TCATCATAGCTGTGCTGATG;
 20 AGAACATGTGATCATCCAGGC; CCAACTATACTCCGATCACCAAT;
 21 TGACAGTGACACTGATGGTGCTG; GGGGACACCCGACCACGTTTC;
 22 TGCAGACACAACTACGGGGTTG; TGGCTGAGGGCAGAGACTCTCCC;
 23 TGCTACTTCACCAACGGGAC; GGTGTGCACACACAACTAC;
 24 AGGTATTTTACCCAGGGACCAAGAGAT;
 25 ATGTAAAATCAGCCCGACTGCCTCTTC;
 26 GCCTCGTGCCCTTATGCGTTTGCCCTCCT;
 27 TGAGGTTAATAAACTGGAGAA; GAGAGTGGCGCCTCCGCTCAT; and
 28 GAGTGAGGGCTTTGGGCCGG.

- 29 30. An HLA Class I locus-specific primer pair.
- 30 31. An HLA Class II locus-specific, intron-spanning
- 31 primer pair.
- 32 32. A DNA sequence defined by an HLA locus-specific
- 33 primer pair.
- 34 33. A kit comprising at least one HLA locus-specific
- 35 primer pair in a suitable container, wherein said
- 36 HLA locus-specific primer pair is selected from

1 the group consisting of an HLA Class I locus-
2 specific primer pair and an HLA Class II locus-
3 specific, intron-spanning primer pair.
4 34. The kit of Claim 33 additionally comprising at
5 least one endonuclease that cleaves a DNA sequence
6 defined by said HLA locus-specific primer pair
7 into a plurality of cleaved sequences of
8 sufficiently different lengths to distinguish
9 between alleles of said HLA locus.
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